Isolation and Identification of Nonstarter Lactic Acid Bacteria from Traditional Baramily Cheese

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Received 25 November, 2020 Accepted 18 February, 2021

Abstract

Nonstarter lactic acid bacteria (NSLAB) have an important role in quality and safety of traditional Baramily cheese (Domiati cheese related type). Therefore, the objective of this study was to isolate and identify NSLAB with potential technological features from traditional Baramily cheese. Thirty-three samples of Baramily cheese randomly collected from retails in Cairo metropolitan area. The samples were characterized by physiochemical, textural profile and microbiological analysis. Ninety presumptive NSLAB (30 Lactobacillus spp. and 35 Enterococcus spp.) strains were isolated on MRS and Kenner-Faecal (KF) Streptococci media; and were characterized for growth temperature, salt tolerance and milk coagulation. All presumptive NSLAB isolates were tolerant to 6.5 % NaCl. Of them, 40 isolates were tolerant to 10.0 % NaCl including 16 presumptive Lactobacillus spp., and 24 presumptive Enterococcus spp. isolates. based on the results, 11 representative isolates with potential technological features were selected for genetic identification using 16S rRNA technique, then were confirmed for growth and acidity development in skim milk within 48 h, and were tested for antimicrobial activity against some food spoilage and pathogenic microorganisms. Both Lb. rhamnosus and Lb. plantarum were confirmed as the isolates with high activity in milk. Ent. durans and Ent. faecalis exhibited antimicrobial activity against Enterobacter aerogenes and E. coli. However, Lb. plantarum exhibited antimicrobial activity against both Enterobacter aerogenes, E. coli and Ps. aeruginosa. Listeria. monocytogenes, S. typhimurium, and Campylobacter. jejuni showed significant resistance to all tested NSLAB isolates. They can be concluded that the identified NSLAB isolated can be used to standardized and improve the quality and safety of Baramily cheese and other types related to Domiati cheese.

Keywords: Baramily; Cheese; NSLAB; Isolation; Identification.

1 Introduction

Lactic acid bacteria (LAB) have an essential role in the quality and safety of cheese and fermented food (Bylund 1995). They are used in cheese production as starter cultures, and adjunct cultures. They may present in cheese as contaminants from processing equipment environment and personnel. Nonstarter lactic acid bacteria (NSLAB) include four main
groups of bacteria: mesophilic lactobacilli, Pediococci, Enterococci and Leuconostoc, which are not part of cheese cultures (Casey et al 2006). Most artisanal and traditional cheeses contain at least one of these four groups, depending on cheese variety, processing steps and ripening conditions (Beresford 2003). NSLAB can dominate the cheese microflora and contribute to the quality and safety of the ripened cheese. Also, NSLAB have an important protective role against food pathogens and spoilage bacteria through the production of different metabolites including bacteriocins, organic acids, acetaldehyde, diacetyl and hydrogen peroxide (Essid et al 2009). The facultatively heterofermentative species of Lactobacilli including Lb. plantarum, Lb. casei, L. paracasei, Lb. rhamnosus and Lb. pentosus are frequently isolated from cheese (Settanni and Moschetti 2010). Also, the genus Enterococcus, especially Ent. durans, Ent. faecium and Ent. faecalis were frequently isolated and identified from many types of traditional cheese manufactured from raw and pasteurized milk (Cogan et al 2007).

Domiati cheese is a popular Egyptian soft white cheese. Sodium chloride is normally added to the milk before renneting in variable large quantities depending on milk quality and season. Tallaga (refrigerated in Arabic), Baramily (barrel in Arabic) and Double Cream are cheese types closely related to Domiati cheese. They mainly matured and stored in metal tins without brine under refrigeration for up to 3 months before consumption. These cheese types are manufactured with lower salt, rennet and renneting temperature lower than those of traditional Domiati cheese. The ripened cheeses have clean acid flavor and smooth creamy body and texture. NSLAB have been isolated and identified from Domiati cheese including Ent. faecalis, Ent. faecium and Lb. plantarum (El-Zayat et al 1995). During the late aging stages of Domiati cheese, Lactobacillus spp. become dominant (Shehata et al 1984). The isolated cocci strains from ripened Domiati cheese are usually salt-tolerant Enterococci (Hematat et al 1998).

Isolation and screening of LAB from natural environments is are the most effective strategy to select LAB strains with potential features to improve cultures used in the production of cheese and fermented milk (Ibourahema et al 2008). Therefore, the objective of this research was to isolate, characterize and identify NSLAB with potential technological features from traditional Baramily cheese.

2 Materials and Methods

2.1 Cheese sampling

Thirty-three samples of each ripened and fresh traditional Baramily cheese were randomly collected from retails in Cairo metropolitan area. The samples were handled and kept refrigerated (3°C) until analysis.

2.2 Physicochemical analysis

The pH value of cheese samples was determined by mixing 25 g cheese with distilled water at a ratio of 1:1 in stomacher (Steward 400, UK) before measuring by a pH meter (Rehman and Fox 2002). Fat content was determined using Gerber method as described by (IDF 1997) and protein content was determined using Kjeldahl method (Fox et al 2000). Salt content was determined using Mohr method (IDF 1988) and Total solids were determined according to AOAC (2012).

2.3 Texture profile analysis

Quadrilateral samples were prepared with dimensions of 10x10x10 mm. Cheese cubes were cut from a deep of 12 mm of surface to eliminate the effect of surface dryness. Textural profile including hardness, cohesiveness, springiness, adhesiveness, and chewiness were determined with a Texture Analyzer (model TMS-Pro, Food Tech. Co., USA). A two-bite penetration test was performed with TA60 degree cone and Perspex probe for cheese operated at a crosshead speed 50 mm/sec. Calculations described by Bourne (2002) were used to obtain the texture profile parameters.
2.4 Microbiological analysis

Using sterile cheese triers, 10 g cheese samples were cut under aseptic conditions, were decimally diluted in sodium citrate solution (2%), then, were homogenized in a stomacher (Steward 400, UK) at normal speed for 2 min. Further dilutions were decimally prepared with Maximum Recovery Diluant (MRD: 8.5 g NaCl and 1 g peptone/l). Dilutions were plated on specific media to count, detect and isolate target microorganisms. Lactobacilli and Enterococci were enumerated on MRS and KF Streptococci agar (Oxoid) with 2,3,5-Triphenyltetrazolium chloride 1% (TTC) as a supplement and incubated at 37°C and 42°C for 24-48 h, respectively (Pisano et al 2006). Total mesophilic bacterial counts were determined on plate count agar (Oxoid) incubated at 30°C for 72 h (ISO 4833-1:2013). Coliform bacteria were enumerated on Violet Red Bile Lactose (VRBL) agar (Oxoid) incubated at 37°C for 22 to 26 h (ISO 4832, 2006). Yeast and Molds were counted on Oxytetracycline Glucose Yeast Extract Agar (Oxoid) with incubation at 25°C for 5 to 7 days according to ISO 21527-1:(2008).

According to the method described by ISO 6888-1 (2003), Staphylococcus aureus was enumerated on Baird–Parker agar (Oxoid) with incubation at 37°C for 24 h. In accordance with ISO 6579-1 (2017), Salmonella spp., was detected using Rappaport–Vassiliadis (RVS) broth (Oxoid) with incubation for 24 h at 41.5°C. Then, plates of Xylose Lysine Deoxycholate (XLD) agar (Oxoid) were streaked RVS incubated broth, then, incubated for 24 h at 37°C.

2.5 NSLAB isolation and preservation

Presumptive Enterococci isolation was performed on KF Streptococci agar (Oxoid) at pH 7.1, which incubated for 48 h at 42°C. Isolation of lactobacilli was performed on MRS agar (Oxoid) at pH 5.4, which incubated for 48 h at 37°C in a carbon dioxide incubator (5%) according to Pisano et al (2006).

2.6 NSLAB characterization

The ninety presumptive NSLAB isolates were preliminary characterized for ability to coagulate milk, grow at different temperatures (10°C and 45°C) and salt tolerance (6.5 and 10% NaCl) in MRS and KF Streptococci broth media (OXOID) according to Whittenbury (1964) and Yavuzdurmaz (2007).

2.7 NSLAB identification

Eleven selected isolates with potential technological role in cheese quality and safety were identified using 16S rRNA sequence analysis methods by genomic as following:
1) DNA extraction was performed using genomic DNA purification kit (Thermo K0721, Gene Jet™).
2) PCR was performed using Maxima Hot Start PCR Master Mix (Thermo K1051).
3) PCR was clean up to the PCR product using GeneJET™ PCR Purification Kit (Thermo K0701).

PCR product sequencing was performed by ABI 3730xl DNA sequencer with forward and reverse primers (GATC Co., Germany).

2.8 Activity of identified NSLAB isolates in milk

Identified NSLAB isolates were assessed for activity in skim milk (growth as log cfu/ml and pH reduction) at 37°C for 48 h.

2.9 Antimicrobial activity of identified NSLAB isolates

Antimicrobial activity of the identified NSLAB isolates was assayed in Cell-Free Filtrates (CFF) using the paper disc diffusion
method against some food spoilage and pathogenic microorganisms including Enterobacter aerogenes EMCC 30053, E. coli NRRL 3008, L. monocytogenes EMCC 19116, S. typhimurium. ATCC 25566, Ps. aeruginosa ATCC 27853, C. jejuni EMCC 6214 and Staph. aureus EMCC 6538 according to Albano et al (2007) and Buntin et al (2008). All indicator strains were obtained from Cairo Microbiological Resource Center (MIRCEN), Faculty of Agriculture, Ain Shams University.

2.10 Statistical analysis

The statistical analysis of treatment effects was calculated through a General Linear Model (GLM), separating the three replicates via Duncan’s Multiple Range Test (MRT) at P≤0.05 (SAS, 2009).

3 Results and Discussions

3.1 Physiochemical analysis

Data in (Table 1) show the physiochemical composition of Baramily cheese samples. Total solids were 42.34 ±1.98 in fresh cheese samples while it were 46.05 ±0.65 in ripened samples. These results were complying with the limits required by the Egyptian standards (EOS, 2005), which should be 40% at minimum.

Fresh cheese samples contained 19.50 ±0.67% fat and ripened cheese samples contained 21.60 ±0.22 as a result of decreased moisture and increased total solids due to whey syneresis during the ripening period. The Egyptian standards do not set a limit for fat content, but a limit for fat in dry matter (60% at minimum), which was exceeded according to the determined fat and solids contents. These results were higher than results reported by Idris (1989) who found that fat content of Domiati cheese ranged from 14.0 to 15.31 with a mean of 14.46%.

In the same trends with total solids and fat content, protein content was 14.33 ±0.46 in fresh cheese while it was 17.17 ±0.15 in ripened cheese samples. These results were remarkably above the minimum protein content required by Egyptian standards (EOS 2005), which should be 10% at least and the results reported by Abou-Dawood et al (2005) found that total protein of Domiati cheese ranged from 5.35 to 12.5 with a mean of 7.89%. However, the results compared to those reported by Dabiza et al (1999) who reported that TP of soft white cheese ranged from 17.7 to 20.5%. The variation in protein content depended on several factors such as milk composition, process efficiency, moisture content, acidity development and the rate of whey syneresis (Idris 1989).

Table 1. Physiochemical analysis of Baramily cheese samples.

<table>
<thead>
<tr>
<th>Physiochemical parameter</th>
<th>Mean±SE²</th>
<th>Egyptian Standard *</th>
<th>Fresh</th>
<th>Ripened</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (%)</td>
<td>57.66 ±1.98 a</td>
<td>53.95 ±0.65 b</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>Total Solids (%)</td>
<td>42.34 ±1.98 b</td>
<td>46.05 ±0.65 a</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Fat (%)</td>
<td>19.50 ±0.67 b</td>
<td>21.60 ±0.22 a</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>Protein (%)</td>
<td>14.33 ±0.46 b</td>
<td>17.17 ±0.15 a</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Salt (%)</td>
<td>09.15 ±0.29 a</td>
<td>07.24 ±0.16 b</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>05.63 ±0.07 a</td>
<td>04.45 ±0.02 b</td>
<td>--</td>
<td></td>
</tr>
</tbody>
</table>

¹SE: Standard Error. ²Egyptians Standard: 3- 1008/2005. Data is a mean of 33 samples. Means in the same row with the same superscript letters are insignificantly different (P ≤0.05).

AUJASCI, Arab Univ. J. Agric. Sci., 29(1), 2021
Contrary with solids, fat and protein, salt content was 09.15 ±0.29 in fresh samples while it was 07.24 ±0.16 in ripened samples. The ripened cheese complied with the salt limits required by the Egyptian standards (EOS 2005), which should not exceed 9%. These results were higher than the results reported by Abou-Dawood et al (2005) who found that salt content of Domiati cheese ranged from 3.0 to 6.0 with a mean of 4.2%, while they were lower than the results as reported by Hofi et al (1975) who found that salt content of Domiati cheese ranged from 16.26 to 20.97 with a mean of 18.22%. The early high salting level in Domiati cheese and related types acted as a preventive control for potential high microbial counts in milk and processing environment.

The mean value of pH in fresh cheese samples was 05.63 ±0.07, which was compared to the results reported by Idris (1989) who found that pH values of Domiati cheese ranged from 5.6 to 5.8 with a mean of 5.72. In the ripened cheese samples, pH fell to 04.45 ±0.02 due to the developed acidity by lactic microflora. This acidic pH was reported by Hofi et al (1975) who found that pH values of Domiati cheese ranged from 4.5 to 4.8 with a mean of 4.6. The relatively decreased pH of ripened cheese samples is attributed to cheese manufacturing from raw milk with high level of natural microflora.

3.2 Texture Profile analysis

The data in (Table 2) presents the texture profile analysis of fresh and ripened Baramily cheese samples. There were significant differences between fresh and Baramily cheese in all parameters including hardness (N), springiness (mm), adhesiveness (mj), cohesiveness and chewiness (mj). hardness (N), adhesiveness (mj) and cohesiveness significantly increased after cheese ripening. Contrary, both springiness (mm) and chewiness (mj) significantly decreased. The increase of hardness in ripened cheese may have been related to acid development and whey syneresis concomitant with the decrease in moisture during storage (Souza and Saad, 2009). Ahmed et al (2005) and Souza and Saad (2009) reported a gradual decrease in the adhesiveness values of traditional soft cheese during the storage period. They suggested these results were attributed to high fat value in cheese which was not sufficiently retained in the protein matrix. Contrary, the decrease in chewiness may have been related to proteolysis and lipolysis.

### Table 2. Texture profile analysis of Baramily cheese

<table>
<thead>
<tr>
<th>Texture Profile Parameters</th>
<th>Mean±SE(^1)</th>
<th>Fresh</th>
<th>Ripened</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hardness (N)</td>
<td>10.95±0.87 b</td>
<td>15.93±1.01 a</td>
<td></td>
</tr>
<tr>
<td>Springiness (mm)</td>
<td>11.19±0.42 a</td>
<td>05.14±0.34 b</td>
<td></td>
</tr>
<tr>
<td>Adhesiveness (mj)</td>
<td>01.7±0.10 b</td>
<td>01.73±0.21 a</td>
<td></td>
</tr>
<tr>
<td>Cohesiveness</td>
<td>00.64±0.03 b</td>
<td>00.97±0.04 a</td>
<td></td>
</tr>
<tr>
<td>Chewiness (mj)</td>
<td>78.61±2.26 a</td>
<td>48.81±2.03 b</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) SE: Standard Error. Data is a mean of 33 samples. Means in the same row with the same superscript letters are insignificantly different (P ≤0.05).

3.3 Microbiological analysis

Traditional Domiati cheese and Baramily are still widely made of raw milk lacking compliance with Egyptian standards (EOS 2005), which require milk pasteurization before processing and distribution. As shown in (Table 3), both fresh and ripened samples were highly populated with NSLAB, despite of a significant decrease was observed in ripened cheese. The high counts of NSLAB may be attributed to the half level salt added in Baramily cheese compared to Domiati cheese. The hygiene indicator microorganisms including total bacteria, coliform bacteria, and yeast & moulds counts were significantly high in fresh samples. Significant decreases were determined for all hygiene indicator microorganisms in ripened cheese samples. Moreover, coliform bacteria were eliminated. Regarding food pathogens, both Staph. aureus and S. typhimurium did not survive in ripened cheese samples. These results agreed to the results reported by Sayed et al (2011), who examined...
Table 3. Microbial counts (log cfu/g) in Baramily cheese samples

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Mean±SE: log cfu/g</th>
<th>Egyptian Standards Limits cfu/g or 25 g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fresh</td>
<td>Ripened</td>
</tr>
<tr>
<td>Lactobacillus spp.</td>
<td>0.738 ±0.43 a</td>
<td>6.08b ±0.14 b</td>
</tr>
<tr>
<td>Enterococcus spp.</td>
<td>0.546 ±0.30 a</td>
<td>4.59±0.21 b</td>
</tr>
<tr>
<td>TBC</td>
<td>0.78 ±0.43 a</td>
<td>6.34 ±0.14 b</td>
</tr>
<tr>
<td>Total Coliform</td>
<td>0.49 ±0.35 a</td>
<td>0.00 b</td>
</tr>
<tr>
<td>Yeast and Moulds</td>
<td>0.247 ±0.50 a</td>
<td>2.85 ±0.16 b</td>
</tr>
<tr>
<td>Staph. aureus</td>
<td>0.029 ±0.01 a</td>
<td>0.00 b</td>
</tr>
<tr>
<td>Salmonella spp.</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

1 SE: Standard Error. 2 Egyptian Standards: 3-1008/2005. Data is a mean of 33 samples. Means in the same row with the same superscript letters are insignificantly different (P ≤0.05).

Domiat, Bramily, Fayomi and Tallaga cheese samples for aerobic plate count, thermoduric, psychrotrophs, Enterococci, coliforms, fecal coliforms, E. coli, Staph. aureus, yeasts and molds and anaerobes. They reported that microbiological counts exceeded the maximum limits set by the Egyptian Standards. These results suggested improving hygiene practices along milk production and processing chain with a cheese ripening period for 90 day as a control measure to reduce microbial food pathogens to acceptable levels.

3.4 NSLAB isolation and characterization

Ninety presumptive NSLAB isolates were confirmed as a gram-positive and catalase negative strains (35 presumptive Lactobacillus spp. and 55 presumptive Enterococcus spp. isolates) as shown in (Table 4).

All presumptive NSLAB isolates were tolerant to 6.5% NaCl salt in MRS and KF Streptococci broth media. Among the presumptive NSLAB, 40 isolates were tolerant to 10.0% NaCl salt including 16 presumptive Lactobacillus spp., and 24 presumptive Enterococcus spp. isolates.

NSLAB isolates exhibited variable ability to grow and develop acidity in milk. The results indicated that presumptive lactobacilli were fast acid producer (within 6 h) than presumptive Enterococci. With continued incubation to 24 h, the different isolates exhibited acidity development in milk. These results are in agreement with previous studies reported that isolated cocci strains from mature Domiat cheese were mainly salt-tolerant enterococci (Hemati et al 1998), but other microorganisms that have been found in the product including Ent. faecalis, Ent. faecium and Lb. plantarum (El-Zayat et al 1995).

3.5 NSLAB identification

Table 5 shows molecular identification of 11 NSLAB isolates at the species level based on partial sequence of 16S rRNA gene. They were Ent.durans (NSLAB 1), Ent.faecalis (NSLAB 2-6), Lb. paraplantarum (NSLAB 7), Lb. plantarum (NSLAB 8-10) and Lb.rhamnosus (NSLAB 11).

Isolated Enterococcus and Lactobacillus from cheese samples can grow in various environments such as acid, aw, salt and temperature. Therefore, it is logic to consider that NSLAB isolated from Baramily cheese is well adapted to cheese processing and ripening conditions. Subsequently, they dominant in final cheese product.

Enterococci, particularly Ent. faecalis and Ent. Faecium often found as NSLAB in different types of traditional cheeses manufactured from raw and pasteurized milk (Cogan et al 2007). The occurrence of Enterococci in cheese may be ascribed to inefficient pasteurization and post pasteurization contamination from unhygienic contact surfaces and environments (Robinson 1990). Food contamination
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Table 4. Preliminary Characterization of presumptive NSLAB isolates from Baramily cheese

<table>
<thead>
<tr>
<th>Presumptive NSLAB Isolates</th>
<th>Growth in 6.5% Salt</th>
<th>Growth in 10% Salt</th>
<th>Milk coagulation time (h)</th>
<th>Growth Temp. °C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Media</td>
<td>Shape</td>
<td>No %</td>
<td>No %</td>
</tr>
<tr>
<td></td>
<td>MRS</td>
<td>Roads</td>
<td>35</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>KF Streptococci</td>
<td>Cocci</td>
<td>55</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 5. Identification of NSLAB isolated from Baramily Cheese by 16S rRNA sequencing

<table>
<thead>
<tr>
<th>NSLAB No.</th>
<th>Identification</th>
<th>Accession Number</th>
<th>Ref. Strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ent. durans</td>
<td>NR_113257</td>
<td>JCM 8725</td>
</tr>
<tr>
<td>2-6</td>
<td>Ent. faecalis</td>
<td>NR_113901</td>
<td>NBRC 100480</td>
</tr>
<tr>
<td>7</td>
<td>Lb. paraplanatarum</td>
<td>NR_025447</td>
<td>DSM 10667</td>
</tr>
<tr>
<td>8-10</td>
<td>Lb. plantarum</td>
<td>NR_104573</td>
<td>CIP 103151</td>
</tr>
<tr>
<td>11</td>
<td>Lb. rhamnosus</td>
<td>NR_113332</td>
<td>NBRC 3425</td>
</tr>
</tbody>
</table>

with enterococci usually attributed to poor microbiological quality of ingredients and unhygienic environment (Franz et al 1999) despite of it has been proposed to use Enterococci as a culture for some cheese types (Garde et al 1997).

Cogan et al (2007) reported that dominant NSLAB in raw or pasteurized cheese are frequently facultative homofermentative lactobacilli, which mainly produce lactic acid from lactose. They mainly include Lb. plantarum and Lb. casei. Luiz et al (2016) isolated and identified Lactobacillus, Enterococcus, Pediococcus, and Lactococcus from Brazilian Minas artisanal cheese. Samelis et al (2010) investigated the secondary microflora of Graviera cheese and they reported that identified LAB species were Lb. casei Lb. paracasei, Lb. plantarum, Ent. faecium, S. thermophilus, and Lc. Lactis. Also, Wassie and Wassie (2016) identified a total of 83 LAB isolates belonged to six genera including Lactobacillus, Lactococcus, Leuconostoc, Streptococcus, Pediococcus and Enterococcus.

3.6 Activity of NSLAB isolates in skim milk

Fig 1 illustrates the growth and acidity development (pH reduction) of Ent. faecalis and Ent. durans isolates in skim milk at 37°C for 48h. The results indicated that Ent. faecalis had higher and rapid growth and pH reduction than Ent. durans. Ent. faecalis had achieved a maximum count (9.89 log cfu/ml) at 24 h with reduced pH to 4.71. Declined growth associated with a final pH reduction to 4.58 was determined after 48h of incubation.

As illustrated in (Fig 2), the maximum growth among all Lactobacillus isolates in skim milk at 32°C for 48 h was determined for Lb. rhamnosus within 12 h of incubation (Fig 2- C). Also, the same isolate exhibited maximum ability to develop acidity and reduce skim milk pH to the minimum compared to other L. isolates. Also, Lb. plantarum proved a good ability to grow in skim milk and coagulate the milk after 12 h of incubation and had attained the stationary phase after 18h of incubation at 32°C. Whereas, Lb. paraplanatarum
Fig 1. Growth and pH reduction of *Ent. faecalis* (a) and *Ent. durans* (b) isolates in skim milk at 37°C for 48h.

Fig 2. Growth and pH reduction of *Lb. plantarum* (a), *Lb. paraplantarum* (b) and *Lb. rhamnosus* (c) isolates in skim milk at 32°C for 48h.
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Table 6. Antimicrobial activity of identified NSLAB isolates against some hygiene indicator and pathogenic microorganisms

<table>
<thead>
<tr>
<th>NSLAB Isolates</th>
<th>Inhibition Indicator Microorganisms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ent. aerogenes DSM 30053</td>
</tr>
<tr>
<td>Ent. Durans</td>
<td>+</td>
</tr>
<tr>
<td>Ent. Faecalis</td>
<td>+</td>
</tr>
<tr>
<td>Lb. paraplanatarum</td>
<td>ND</td>
</tr>
<tr>
<td>Lb. plantarum</td>
<td>+</td>
</tr>
<tr>
<td>Lb. rhamnosus</td>
<td>ND</td>
</tr>
</tbody>
</table>

³ND: Non-Detected inhibition (zone diameter less than 1 mm).

3.7 Antimicrobial activity of NSALAB isolates

Table 6 shows that both Ent. durans and Ent. faecalis exhibited antimicrobial activity against tested Enterobacter aerogenes and E. coli, while, there was no antimicrobial activity against Listeria monocytogenes, S. typhimurium, Ps. aeruginosa, Staph. aureus or Campylobacter jejuni. Among the identified Lactobacillus isolates, Lb. paraplanatarum did not show any antibacterial activity against all tested microorganisms, but Lb. plantarum exhibited antimicrobial activity against both Ps. aeruginosa, Enterobacter aerogenes and E. coli. Also, Lb. rhamnosus had antimicrobial activity against Ps. aeruginosa, E. coli, and Staph. aureus. Finally, Listeria monocytogenes, S. typhimurium, and Campylobacter jejuni showed resistance to all tested NSLAB isolates.

Our findings agreed to the results reported by many studies which confirmed the inhibiting activities of LAB against food pathogens and spoilage bacteria. Enterococci produce bacteriocins that inhibit some food pathogens including Listeria monocytogenes, Staph. aureus, V. cholerae, Clostridium spp. and Bacillus spp. (Giraffa 2003). The genus Lactobacillus exhibited a wide range of antimicrobial activity that associated with antimicrobial metabolites (Essid et al 2009). Therefore, it is reported that some Lactobacillus species can be used as adjunct cultures in cheese and fermented dairy products (Amin et al 2009).

4 Conclusions

Traditional Baramily cheese samples collected and characterized in this study complied with the Egyptian standards regarding the chemical composition. To ensure cheese
safety, it is suggested to control raw milk Bar- amily cheese by the 90-day ripening rule with hygiene control in milk production and cheese processing. Eleven isolates were identified as Ent. Durans (1), Ent. faecalis (5), L. para- plantarum (1), L. plantarum (3), and L. rham- nosus (1), which all were confirmed active in skim milk, and some exhibited antimicrobial activity against tested food spoilage and pathogenic microorganisms. Finally, our findings suggested that the identified NSLAB isolated can be utilized as adjunct cultures to standardized and improve the quality and safety of Bar- amily cheese and other related Domiati cheese types.

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Isolation and Identification of Nonstarter Lactic Acid Bacteria from Traditional Baramily Cheese


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عزل وتعريف بكتريا غير بادئ حمض اللاكتيك من الجبن البراميلي التقليدي

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Received 25 November, 2020 Accepted 18 February, 2021

حيث النمو على درجات حرارة مختلفة، وتحمل الملح، والقدرة على تكييف الفوتوحموضة، تم تعرف عدد 11 عزلة ذات مميزات تكنولوجية محتملة، وتاثيراً باستخدام تقنية RNA 16s، ثم تم تأكيد قدرتها على النمو وتكوين الحموضة في اللبن الفرز خلال 48 ساعة. Ent. durans (1)، Ent. faecalis (5)، Lb. paraplantarum (1)، Lb. plantarum (3)، و Lb. rhamnosus (1)، والتي تمت تشبيهها جميعاً في اللبن الفرز وأظهرت نشاطاً مضاداً لبعض الميكروبى منغضاً لبعض الميكروبى منغضاً لبعض الميكروبى منغضاً لبعض الميكروبى منغضاً لبعض الميكروبى منغضاً لبعض الميكروبى منغضاً لبعض الميكروبى منغضاً لبعض الميكروبى منغضاً لبعض الميكروبى منغضاً لبعض الميكروبى منغضاً لبعض الميكروبى منغضاً لبعض الميكروبى منغضاً لبعض الميكروبى منغضاً لبعض الميكروبى منغضاً لبعض الميكروبى منغضاً لبعض الميكروبى منغضاً لبعض الميكروبى منغضاً Lactobacillus spp و Enterococcus spp، وذلك على بيئة MRS و KF Streptococci. و

الموجز

تلعب بكتريا غير بادئ حمض اللاكتيك (NSLAB) دورًا هامًا في جودة وسلامة الجبن البراميلي التقليدي وهو من أصناف الجبن الدمياطى. لذلك كان الهدف من هذه الدراسة هو عزل وتعريف بكتريا غير بادئ حمض اللاكتيك ذات المميزات التكنولوجية المحتملة من الجبن البراميلي التقليدي حيث تم تجميع عدد 33 عينة من الجبن البراميلي عشوائياً من متاجر التجزئة بمنطقة القاهرة الكبرى، وتم توصيف العينات بالتحليل الفيزيوكيميائي، والنانسي والميكروبيولوجي. وتم عزل عدد 90 سلالة محتملة لبكتريا غير موجودة ببادئ، بكتريا حمض اللاكتيك (30) Entercoccus spp. و 35 Lactobacillus spp، وذلك على بيئة MRS (Enterococcus spp. المتخصصة وتم تقييمها من